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Effects of Rivaroxaban Therapy on ROTEM Coagulation Parameters in Patients with Venous Thromboembolism**

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Rivaroxaban (Xarelto) does not require routine coagulation monitoring; however, in certain clinical situations (overdose, drug accumulation, urgent surgery) measurement of its plasma concentration is highly recommended. Currently, there is no single hemostasis test that shows a direct correlation between rivaroxaban plasma levels and anticoagulant efficacy.

Objectives. This study was intended to assess the value of ROTEM in determining rivaroxaban administration.

Material and Methods. Thirteen patients with venous thromboembolism and 13 healthy volunteers were compared with regard to certain ROTEM parameters and anti-FXa activity. The tests were done before the administration of 20 mg rivaroxaban (i.e. 24 h after previous administration) and 2.5 h afterwards.

Results. The study group demonstrated residual activity of rivaroxaban in plasma (20 ± 11.3 ng/mL) 24 h following the previous administration, which did not cause marked changes in clotting assays compared to controls. In the group, 2.5 h after rivaroxaban administration, prolongation of PT (PTratio 1.51 ± 0.22), APTT (APPTratio: 1.30 ± 0.14) and ROTEM CT (CTratio – EXTEM: 2.45 ± 1.06, CTratio – INTEM: 1.32 ± 0.21) were observed. The cut-off values for particular tests were created to determine if the patient had achieved desirable anticoagulant effect after rivaroxaban administration. The mean anti-FXa values were significantly lower in patients before rivaroxaban dosing than after.

Conclusions. PT demonstrated better diagnostic value than APTT in rivaroxaban administration. The ROTEM clotting time (CT) according to EXTEM may be used to determine the anticoagulation effect of rivaroxaban, but is not sensitive enough to measure the residual activity of this drug (**Adv Clin Exp Med 2015, 24, 6, 995–1000**).

Key words: ROTEM, anti-FXa method, PT, APTT.

Rivaroxaban (Xarelto), a direct, specific Factor Xa (FXa) inhibitor, is nowadays broadly used for the prevention of stroke and systemic embolism in non-valvular atrial fibrillation, as well as the prevention and treatment of venous thromboembolism (VTE) in various clinical settings [1–3]. Although rivaroxaban does not require routine coagulation monitoring, measurement of its plasma concentration is highly recommended in certain situations, including overdoses, drug accumulation or during the period before urgent surgery [4, 5]. This is especially important, since, as with other new oral anticoagulants (NOACs), no specific antidote exists for the reversal of its anticoagulant effect in the case of severe bleeding [6].

It has to be emphasized that no single laboratory hemostasis test has shown any direct correlation between rivaroxaban plasma levels and either anticoagulant efficacy or the risk of bleeding. Nevertheless, anti-FXa chromogenic assays seem to be better than prothrombin time (PT) assessment for the quantitative measurement of Xarelto plasma

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levels [4]. However, as this assay is time consuming and is not commonly available, PT is still used as a screening test for rivaroxaban measurements in the clinical situations described above.

Rotation thromboelastometry (ROTEM) allows for 'near patient' rapid assessment of clot strength and stability in the perioperative setting, as well as in trauma and septic patients [7–9]. It has been recently shown to identify the prothrombotic state which characterizes the majority of patients with multiple myeloma at the time of diagnosis [10]. The present study was intended to assess the value of ROTEM in the evaluation of rivaroxaban anticoagulant efficacy in patients with VTE. It also attempts to identify possible correlations between certain ROTEM parameters and the results of screening hemostasis tests, as well as with anti-FXa measurements before and 2.5 h after 20 mg oral rivaroxaban administration.

Patients and Methods

Patients

Thirteen patients treated with rivaroxaban for an average of 185 days (range: 32–723 days) due to VTE were enrolled into the study. All gave their informed consent. All patients had normal renal function (GFR; glomerular filtration rate; > 60 mL/min/1.73 m^2) and had not taken other medications with a strong influence on hemostasis for at least 2 weeks before the tests. Other exclusion criteria included known liver disease or alanine aminotransferase > 2 upper limit range, diabetes and surgery in the past month.

In all study participants, screening tests of hemostasis (PT, APTT; activated partial thromboplastin time, fibrinogen concentration), anti-Xa measurements and tromboelastometry tests were performed in two time points: before rivaroxaban administration (24 h after the previous dose) and 2.5 h after the oral dose of 20 mg. The control group consisted of 13 healthy volunteers.

Methods

Citrated samples of blood were collected under standardized conditions and processed for ROTEM measurements within a maximum of 2 h. Platelet poor plasma (PPP) was obtained and stored at –80°C until the anti-Xa test was performed.

PT, APTT and fibrinogen concentration assessments were performed using STA reagents (Diagnostic Stago, Asnière, France). Four commercially available tests (EXTEM, INTEM, FIBTEM and APTEM) were conducted by Rotation Thromboelastometry (Pentapharm GmbH, Munich, Germany, software v. 1.5.3.) to measure coagulation time (CT), clot formation time (CFT), and maximum clot firmness (MCF) according to the instructions provided by the manufacturer. The ROTEM methodology is described in detail elsewhere [9, 11–12]. Inhibition of Factor Xa was performed by chromogenic assay, using BIOPHEN DiXaI, BIOPHEN Rivaroxaban Calibrator and BIOPHEN Rivaroxaban Control reagents (HYPHEN, Biomed, France) according to the manufacturer's instructions.

Statistical Analysis

The *t*-test for dependent samples, the Wilcoxon test and Mann-Whitney *U*-test were used to assess the significance of differences between studied groups. Correlations between variables were measured by the Spearman rank correlation coefficient (r). In all analyses, p values less than 0.05 were considered statistically significant.

Results

The mean values of PT and APTT were markedly higher in patients after rivaroxaban therapy as compared to baseline ($p = 0.000001$ and $p = 0.0015$, respectively), while values of fibrinogen concentration did not differ significantly ($p = 0.66$). To determine which of the two studied screening coagulation tests was more prolonged after rivaroxaban administration, the PT and APTT ratio were calculated as follows: PT ratio = PT 2.5 h after administration**:** PT before administration; APPT ratio = APPT 2.5 h after administration: APPT before administration. This analysis (Table 1) showed that PT demonstrated greater prolongation than APTT after rivaroxaban administration.

ROTEM

According to all studied ROTEM tests, CT readings were found to be markedly higher in the group of patients after rivaroxaban dosing compared to baseline. In contrast, no significant differences were observed between patients before rivaroxaban dosing and controls (Table 2). After calculation of CT ratios for EXTEM and INTEM tests (in the similar way as for PT and APTT ratios) it turned out that CT-EXTEM was a subject of greater prolongation than CT-INTEM after rivaroxaban administration. However, it has to be emphasized that the results of CT-EXTEM showed higher variability (CV; coefficient of variation – 43.56%) as compared to CT-INTEM (CV

Table 1. Prothrombin time ratio and activated partial thromboplastin time ratio 2.5 h after 20 mg oral rivaroxaban administration

Parameter	Mean (95% confidence interval)	SD	CV (%)	p *t*-test for dependent samples
PT ratio	1.51 (1.37–1.64)	0.22	14.84	0.0122
APTT ratio	1.31 (1.22–1.40)	0.14	10.85	

SD – standard deviation, CV – coefficient of variation.

Table 2. Coagulation time (CT) in studied groups of patients and in controls

Parameter		Mean (95% confidence interval)	SD	CV	Median (Q25 – Q75)	p[1)]	p[2)]
EXTEM							
CT (s)	control	57.2 (50.5–63.8)	11.0	19.3	57.0 (51–63)	0.3051	
	before	77.2 (49.7–104.6)	45.4	58.9	69.0 (44–88)		0.0015*
	after	178.5 (106.8–250.1)	118.5	66.4	145.0 (114–186)		
INTEM							
CT (s)	control	197.2 (149.4–245)	79.1	40.1	173.0 (158–187)	0.2486	
	before	200.5 (165.4–235.6)	58.1	29.0	184.0 (179–211)		0.0015*
	after	272.1 (191.2–353)	133.8	49.2	232.0 (206–290)		
FIBTEM							
CT (s)	control	49.5 (43.9–55.2)	9.4	18.9	58 (51–62)	0.0905	
	before	70.5 (50.6–90.3)	32.9	46.7	62 (50–91)		0.0003**
	after	166.3 (106.7–225.9)	98.6	59.3	165 (95–222)		
APTEM							
CT (s)	control	57.1 (52.7–61.5)	7.3	12.7	52 (44–55)	0.2702	
	before	78.8 (54.9–102.8)	39.6	50.2	59 (49–77)		0.0023**
	after	188.8 (105.5–272.2)	138.0	73.1	127 (98–223)		

[1)] Mann-Whitney *U*-test; [2)] * Wilcoxon test; ** t-test for dependent samples; Q25 – first quartile; Q75 – third quartile; SD – standard deviation; CV – coefficient of variation.

– 16.45%). The analysis of other ROTEM parameters revealed that rivaroxaban has no influence on CFT and MCF values besides MCF EXTEM.

Statistical Indicators of Diagnostic Value for PT, APTT, CT-EXTEM and CT-INTEM

The spread of particular results was carefully analyzed and cut-off values for particular tests were created. These cut-off values have the potential to determine whether the patient demonstrated a desirable anticoagulant effect after rivaroxaban administration. In addition, the created cross-tabulation allowed the calculation of sensitivity, specificity, positive and negative predictive values, accuracy and chance for false positive and false negative results (Table 3).

Correlations Between the Results of Screening Coagulation Tests and Selected ROTEM Parameters

Marked positive correlations between PT vs. CT-EXTEM ($r = 0.58$, $p = 0.0018$) and APTT vs. CT-INTEM ($r = 0.8$, $p = 0.000001$) were found in the studied group of patients.

Anti-FXa Method

The mean anti-FXa values were significantly lower in patients before rivaroxaban administration than afterwards (Table 4). Marked positive correlations between anti-FXa vs. PT, anti-FXa vs. APTT, anti-FXa vs. CT-EXTEM and anti-FXa vs. CT-INTEM were found when the entire cohort of patients was analyzed (Table 5). When the patients were divided into specific subgroups, these correlations were only significant in those after rivaroxaban administration (Table 5).

Discussion

As mentioned in the introduction, rivaroxaban therapy does not require routine laboratory inspection, unless within specific clinical settings. Although the anti-FXa chromogenic assays allow for accurate measurements of rivaroxaban anticoagulant activity, these tests are time consuming and cannot be used in such emergency situations such as the occurrence of major bleeding due to an overdose or need for urgent surgery. In contrast, the ROTEM test allows for rapid detection of coagulation abnormalities at the point of care since its results may be obtained within

Table 3. Diagnostic value indicators for PT, APTT, CT-EXTEM and CT-INTEM

Diagnostic parameter	Coagulation time (%)			
	PT	APTT	CT-EXTEM	CT-INTEM
Cut-off	15.6 s	32 s	79 s	200 s
Sensitivity	100.00	100.00	92.31	76.92
Specificity	92.31	84.62	61.54	69.23
Positive predictive value	92.86	86.67	70.59	71.43
Negative predictive value	100.00	100.00	88.89	75.00
Accuracy	96.15	92.31	76.92	73.08
Chance FD	7.69	15.38	38.46	30.77
Chance FN	0.00	0.00	7.69	23.08

FD – false positive, FU – false negative.

Table 4. Anti-FXa results

Parameter		Mean (95% confidence interval)	SD	CV (%)	p *t* –test for dependent samples
Anti-Xa (ng/mL)	before	20.2 (13.3–27.0)	11.3	56.1	0.000051
	after	152.6 (107.2–197.9)	75.0	49.2	

Table 5. Correlations between anti-FXa results and studied coagulation assays

Parameters	Without devision on subgroups	Before drug administration	After drug administration
Anti-Xa vs. PT	0.8906 (p = 0.000000001)	0.3481 (p = 0.2438)	0.7168 (p = 0.0058)
Anti-Xa vs. APTT	0.8758 (p = 0.000000005)	0.0284 (p = 0.9267)	0.7553 (p = 0.0028)
Anti-Xa vs. CT-EXTEM	0.6773 (p = 0.0001)	–0.2091 (p = 0.4929)	0.5798 (p = 0.0378)
Anti-Xa vs. CT-INTEM	0.5600 (p = 0.0029)	–0.2689 (p = 0.3744)	0.5752 (p = 0.0397)

several minutes [13]. Until now, only a few reports have been published concerning the value of the ROTEM device in the context of rivaroxaban therapy [14–16]. However, none of these studies incorporated patients treated for VTE. Our patients received rivaroxaban in daily 20 mg doses for an average of 185 days. Mean rivaroxaban activity measured 24 h after previous drug administration was 20 ng/mL, which was similar to that reported by Mueck et al. [1], while no accumulation of the drug was observed in any patient.

Approximately 2.5 h after drug administration, when the plasma concentration of rivaroxaban was at a maximum, the coagulation time according to EXTEM, INTEM, FIBTEM and APTEM was found to be significantly longer compared to baseline. This contradicts observations made by Casutt et al. [14], who found standard ROTEM tests to be insensitive to direct FXa inhibitors such as rivaroxaban. However, it has to be emphasized that tests performed by Casutt et al. were performed on healthy volunteers and a lower daily dose (10 mg) was used. CT ratio calculation (CT 2.5 h after dosing: CT before dosing) for EXTEM and INTEM tests appears to be a more accurate way of measuring the anticoagulant activity of rivaroxaban in VTE patients. Our findings demonstrate that CT-EXTEM was subject to greater prolongation than CT-INTEM after rivaroxaban administration as compared to baseline, and that rivaroxaban has no impact on any other studied ROTEM parameters beside MCF EXTEM.

In a recently published study, Oswald et al. [16] show increases in CT EXTEM and CT INTEM, decreases in CFT EXTEM and CFT INTEM, as well as increases in MCF EXTEM and MCF INTEM in patients treated with 10 mg rivaroxaban a day for major orthopedic surgery. The tests were performed before surgery and repeated on day 4 after the operation. CT EXTEM was the only variable to increase to a greater degree with rivaroxaban than with enoxaparin, and hence the authors conclude that this test may be particularly useful for detecting treatment with rivaroxaban. The differences between our results and those of Casutt and Oswald may be due to distinct study cohorts (VTE, healthy volunteers, orthopedic patients, respectively), different doses (20 mg vs. 10 mg daily) and different times between drug administration and blood collection (2.5 h vs. 2.5 vs. 14 h, respectively).

Twenty-four hours after previous 20 mg rivaroxaban dosing, no marked differences were found between the patient group and controls with regard to any studied ROTEM parameters. Only CT FIBTEM showed a slight trend (p = 0.09) toward higher values in patients than healthy volunteers. Since the FIBTEM test is dedicated to measuring coagulation without platelet contribution, it cannot be excluded that such test property would increase its sensitivity toward rivaroxaban. It has to be stressed that nothing is currently known regarding the use of the FIBEM test in healthy volunteers or patients treated with rivaroxaban. Furthermore, our observations need to be confirmed on bigger groups of patients.

Recently, Adelmann et al. [15] demonstrated that a modified ROTEM test (low-tissue factor activated ROTEM) is a very sensitive tool for rivaroxaban detection. The authors observed a strong correlation (r = 0.81) between CT and rivaroxaban plasma concentrations. This confirms our present study results, since they also indicate marked correlations between CT EXTEM/CT INEM and rivaroxaban plasma concentrations as measured by anti-FXa chromogenic assay.

Our findings show that PT is more sensitive than APTT for measuring rivaroxaban, which correlates with previous reports [17, 18]. As expected, positive correlations were observed between PT and CT EXTEM and between APTT and CT INTEM. In patients with high plasma rivaroxaban concentrations, significant correlations were also observed between PT/APTT and anti-FXa activity. It has to be stressed that due to different reagents used for PT determination, there is currently strong need for its standardization in the context of rivaroxaban measurement.

In conclusion, our results demonstrate that the ROTEM clotting time according to EXTEM may

be used to determine the anticoagulation effect of rivaroxaban in patients with VTE. However, the test is not sensitive enough to measure the residual activity of this drug. The usefulness of the ROTEM device for rapid, point-of-care rivaroxaban testing has to be confirmed on larger groups of patients.

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